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Chiral drug bioanalysis with on-column sample-focussing

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The potential of using a combination of SPE followed by direct injection of a large volume of SPE eluant for microbore LC on Hypercarb® using a chiral mobile phase additive as an approach to satisfying the continuing need for effective methods for determining low levels of drug enantiomers in biological fluids was explored.

Hypercarb is very retentive under reversed-phase LC conditions. Accordingly even when a drug is eluted from a solid phase extraction cartridge containing retentive C18 or phenyl-silicas, a large volume of the resultant solution may be injected onto a Hypercarb microbore LC column. This results in the drug being concentrated as a narrow band at the head of the column. By following this procedure it is possible to detect the drug present in very low concentrations. Hypercarb is also a good achiral support material in enantioselective LC employing a chiral mobile phase additive.

It had already been demonstrated that on-column sample focussing could still be obtained and chiral resolution not be compromised when a large volume of a sample solution containing drug in an achiral solvent is injected onto a microbore LC system with a mobile phase containing a chiral selector (Prangle *et al*, 1998). In light of this success it was sought to ascertain whether this approach could fulfil the requirements of a reliable quantitative method and how generally it could be applied. With respect to the set of conditions that had been used in this earlier study, validation parameters, eg linearity $r = 0.985$ for first eluting peak, 0.964 for second eluting peak, were acceptable even although the recovery, 15%, was low.

Subsequent studies were therefore carried out using a range of acidic and neutral drugs, the achiral drugs diclofenac, indomethacin and acemethacin as well as the chiral drugs ketoprofen, and benzoin, to examine the whole question of recovery in this approach. The key to good recovery was found to be in the protein precipitation step. The general protocol was therefore changed from the use of one volume of 10 % v/v solution of perchloric acid to one volume of plasma to the use of two volumes of acetonitrile or methanol to one volume of plasma. This resulted in the need for an extra step of the addition of a specified amount of aqueous acetic acid to the solution for application to the SPE cartridge so that analyte was not lost during the application and wash steps.

With respect to generality of the approach it was found for the set of drugs, as had been found for warfarin, that there was a substantial degree of retention on Hypercarb even when using acetonitrile or methanol containing 10% or less aqueous acetic acid. Therefore, since solvent mixtures of these compositions were strong enough to give complete recovery of all the drugs from phenyl BondElut® cartridges, direct injection of the SPE eluant onto a Hypercarb LC column would result in on-column sample focussing. While clearly the use of dimethylated-β-cyclodextrin as the chiral selector could not be expected to be general, it did give good enantioselectivity for racemic flurbiprofen, $\alpha = 1.20$, and naproxen, $\alpha = 1.19$ (Hypercarb (150 x 1 mm), mobile phase acetonitrile- water- acetic acid-triethylamine (500:500:3:2.5, v/v) containing 20 mM dimethylated-β-cyclodextrin).

It can be concluded then that the use of SPE followed by direct injection of a large volume of SPE eluant for microbore LC on Hypercarb using a chiral mobile phase additive offers an attractive approach to the detection of low levels of drug enantiomers in biological fluids. Especially attractive is the generality of the approach to on-column sample focussing with the result that the only significant remaining challenges in method development for any specific chiral drug would be choice of appropriate SPE wash step, to minimise loss of analyte, and chiral selector.

References

Prangle, A.S., Noctor, T.A.G., Lough, W.J. (1998) Chiral Bioanalysis of Warfarin using Microbore LC with Peak Compression. J.Pharm. Biomed. Anal. 16: 1205-1212